# Probiotic tablet formulations

The present invention relates to the formulation of probiotic micro-organisms in tablet form. Probiotic micro-organisms are conventionally formulated with other nutritionally active materials such as vitamins, minerals, carbohydrates, proteins, co-enzymes, enzymes, plant extracts, trace elements, and/or fats. Whilst many probiotic micro-organisms are quite stable when kept by themselves in a dried form, tablet formulations in which the probiotic micro-organisms are mixed with active ingredients of the above kinds are highly unstable. After even brief storage, the recovery of viable micro-organisms upon rehydration of such mixed formulations will be extremely poor.

US6254886 attempts to address this problem by proposing that the tablet should be in a multilayer form with the probiotic micro-organism being contained in a layer which is free from other nutritionally active materials and which is dry to the extent that its water content is less than 0.1%. Since water is in fact free to move between the different layers of the tablet, this in practice means that the carrier material for all the tablet layers has to be dry to this same extent. Moreover, where large amounts of other active ingredients are present, they too will have to be aggressively dried if the total water content of the probiotic layer is not to rise significantly above the limits set in US6254886.

We have now found that the water content in a storage stable probiotic tablet formulation can be very much higher than is taught in US6254886 provided that care is taken that the water activity is maintained below 0.2 (equivalent to 20% relative humidity) and that the mixing with the probiotic micro-organisms of certain active materials taught to be kept separate from the probiotic micro-organisms in US6254886, is not deleterious and may actually improve the viability of the micro-organisms.

The present invention now provides a probiotic tablet comprising a probiotic micro-organism and other nutritionally active ingredients, the tablet comprising at least two zones, a first of said zones comprising said probiotic micro-organism, and a second of said zones comprising at least one said other active ingredient kept separated from the probiotic micro-organism of said first zone, the water activity in said probiotic micro-organism containing first zone being no greater than 0.2 and the water content of said tablet being no less than 0.2% by weight.

Tablets according to the invention, particularly as exemplified below may be storage stable at a cool temperature (up to 15 °C) or more preferably at room temperature (up to 20 °C or more preferably up to 25 °C) for several months, e.g. for up to one year or more preferably up to 18 months or more preferably two years or more. By 'storage stable' is meant that after a storage period, the number of viable probiotic micro-organisms should not have declined by more than a factor of one thousand, preferably not more than one hundred, more preferably not by a factor of more than 10 e.g.. from $5*10^9$ to $5*10^8$ , or less preferably to $5*10^7$ or still less preferably to $5*10^6$.

According to US 6254886, the presence together with the probiotic micro-organism of other substances valuable in nutritional physiology is deleterious. It is suggested that at best there may be some unidentified active materials that are not deleterious. However, we have found that certain active materials actually improve the stability of the product when they are present in the first zone. In accordance with this, it is preferred that said first zone contains also selenium as a said at least one other active ingredient . Preferably, said first zone contains from 1 to 100 μg , e.g. 5 to 75 μg, more preferably 7.5 to 60 μg, of selenium, per $10^9$ micro-organisms.

The presence of selenium together with the micro-organisms is particularly preferred as we have demonstrated that selenium increases the storage stability of the tabletted micro-organisms. The mechanism responsible for this is at present uncertain. It may be that the selenium exerts a beneficial influence in one or more of several ways including as a growth medium, as a compression distributor, as a stabiliser, as a desiccant or as an antioxidant.

The presence in said first zone of antioxidants generally is also preferred. These include ascorbyl palmitate or other ascorbates, propyl gallates or other – gallates, alpha-tocopherol, magnesium or sodium sulfite, butylated hydroxyanisole or butylated hydroxytoluene.

Certain active ingredients are however deleterious and should preferably be excluded from the first zone. These include iron, vitamin B6, vitamin C, zinc, copper, manganese, chromium, pantothenic acid or its salts, and to a lesser extent vitamin B1, so the first zone is preferably free from amounts of some or all of each of these that are sufficient materially to exert an adverse effect on the stability of the product. Several of these materials are available in a micro-encapsulated form. One way in which such materials may be present in a tablet according to the invention without

their being present in the first zone is for them to be encapsulated, but to be present as micro-particles mixed in to the probiotic micro-organism containing material. If the level of separation imposed by the micro-encapsulation of these materials is not adequate, they may still exert an adverse effect, so we prefer that they should not be mixed into the first zone in micro-encapsulated form, but should be relegated to a more physically distinct and separate macro-region of the tablet, such as a distinct layer. This applies especially to iron and copper.

Encapsulated zinc is better tolerated and can be admixed into the first zone materials.

Vitamin B1 can be present in the first zone in non-encapsulated form without much deleterious effect.

Some benefit may come from having certain encapsulated materials mixed into the first zone. These include micro-encapsulated vitamin B1, micro-encapsulated vitamin B6, micro-encapsulated zinc, micro-encapsulated manganese, micro-encapsulated vitamins A, D, E, B12 and B2.

Said second zone preferably contains as at least one said other active ingredient any one of iron, vitamin B6, vitamin C, zinc, copper, manganese, chromium, and pantothenic acid or a salt thereof. Preferably at least two, more preferably at least four, more preferably at least six and preferably all of these are present.

It is preferred that the tablets of the invention have a multi-layer form comprising two or more layers, one of said layers constituting said first zone and another of said layers constituting said second zone. Additional layers may be present. The layers may be formed one over the other or such that a body of material constituting one of the first and second zones is enrobed by a layer of material constituting the other of said zones.

Where such a two layer structure is used, it is still possible for the layer constituting said first zone to contain in encapsulated form some materials which are required to be kept out of the first zone, but for better separation of the probiotic micro-organisms from these materials it is preferred that they are not present mixed within the first zone layer but are present only in the second zone. This reduces the interface area between zones containing the probiotic micro-organism and these potentially destabilising ingredients. These include particularly iron, encapsulated iron, vitamin B6, vitamin C, zinc, copper, manganese, chromium, pantothenic acid

and its salts, and encapsulated copper and to a lesser degree encapsulated zinc, especially if not strongly encapsulated, and vitamin B1.

On the other hand, it may be acceptable or even beneficial if mixed within the layer constituting the first zone are one, two or any combination of micro-encapsulated vitamin B1, micro-encapsulated vitamin B6, selenium, micro-encapsulated zinc, iodine, micro-encapsulated vitamins A, D, E, B12 or B2, nicotinamide, folic acid, or any of the anti-oxidants mentioned herein.

Summing this up, if one were to categorise other active ingredients likely to be present into three lists: A(aggressive ingredients to be kept well away from the probiotic material, e.g. in a separate layer), B (somewhat aggressive ingredients which are preferably excluded from the first zone, but which may well be tolerated either in the first zone or in micro-encapsulated form surrounded by the first zone) and C (non-aggressive or beneficial ingredients that can be present in the first zone or if encapsulated can be surrounded by the first zone) these lists would be as follows:

List A

iron

Encapsulated Fe

Vitamin B6

Vitamin C

Zinc

Copper

Manganese

Chromium

Calcium pantothenate

Encapsulated copper

List B

Vitamin B1

Nicotinamide

List C

Encapsulated vitamin B1

Encapsulated vitamin B6

Selenium

Encapsulated zinc

Iodine

Magnesium

Encapsulated manganese

Encapsulated vitamin A, D, E, B12, B2

Folic acid

Whilst not as well tolerated as the above ingredients in List C, nicotinamide may be categorised either in List B or in List C as may encapsulated zinc..

Whilst layer structures are preferred, it is permissible for the tablet to have a multitude of granules constituting said first zone surrounded by a matrix, wherein said matrix constitutes said second zone or wherein said matrix also contains a multitude of granules constituting said second zone.

In order to obtain a low water activity in the first zone, the probiotic micro-organism is preferably mixed with a desiccant carrier material serving to reduce the water activity of the zone containing the probiotic micro-organism. Optionally however such a desiccant carrier material serving to reduce the water activity of the zone containing the probiotic micro-organism may be present instead in the second zone. Preferably, such a material is present in both the first and the second zones. The effect of such a desiccant may be to sequester part of the water content of the zone so that it is no longer in the form of free water that can migrate into the probiotic micro-organisms and is therefore prevented from carrying active substances through the cell walls of such organisms. Such desiccants bind water to specific sites so that it is no longer able to act as a solvent. These sites include the hydroxyl groups of polysaccharides, the carbonyl and amino groups of proteins, and others on which water can be held by hydrogen bonding, by ion-dipole bonds, or by other strong interactions. Thus, preferred desiccants include at least one of carboxymethylcellulose, colloidal silica, polyvinylpyrrolidone, starch, gelatine, hydroxypropylcellulose, microcrystalline cellulose, fumed silicon dioxide, sodium croscarmellose, crospovidone, povidone, magnesium aluminium silicate, methylcellulose, sodium alginate, sodium starch glyconate, sodium starch glycolate, gelatine, pregelatinized starch, or sorbitol. The desiccant may be in particular, a

starch selected from corn, rice, or potato starch, a hydrophilic gum, polysaccharide or galactomannan such as pectin, agar, dextran, maltodextrin, carageenan, tragacanth gum, locust bean gum, acacia gum, guar gum, xanthan gum, ghatti gum, alginic acid or sodium alginate, a cellulose derivative such as methyl cellulose, carboxymethylcellulose, sodium starch glycollate, sodium or calcium carboxymethylcellulose, hydroxyethyl methylcellulose, hydroxypropylmethylcellulose, ethylhydroxyethylcellulose, ethylmethylcellulose, hydroxyethylcellulose, cellulose acetate phthalate, or microcrystalline cellulose, silica, aluminium silicate, magnesium silicate, aluminium magnesium silicate, sodium silicate or feldspar, aluminium hydroxide, a protein such as gelatin or casein or a polymer such as acrylate, carboxypolymethylene, a polyalkylene glycol or polyvinylpyrrolidone. Other steps to reduce the amount of oxygen present may be beneficial, including packing under an inert atmosphere such as nitrogen and the use of oxygen barrier packaging materials such as aluminium tubes or high barrier polymers.

The water content of the tablet is at least 0.2% by weight and may be considerably higher. Higher water contents remove the need for aggressive drying of materials which may be sensitive to such a process. It is undesirable that the water content in the tablet is too high as it increases the risk of unforeseen re-crystallisation. Also, it is expensive to remove water. Thus, the water content can be above 0.5% or above 1%, but below 6% more preferably below 5%, or 4%, 3% ,or even 2%. Alternatively, the water content can be above 0.5% or above 1% or 2% , but below 6% more preferably below 5%, or 4%, or 3%. Alternatively, the water content can be above 0.5% or above 1% or 2% or 3%, but below 6% more preferably below 5%, or 4%. The water content can go up to 7% by weight.

At the same time, the water activity is preferably below 0.18, more preferably below 0.15, still more preferably below 0.13, e.g. 0.10, or even 0.08. The water activity may be still lower, e.g. 0.05 or even 0.02. The water activity may lie between 0.2 and any of the foregoing figures or between any two of them.

Each of the foregoing figures for water activity relate to the first zone of the tablet. Normally, following internal equilibration, this will also be the water activity of the tablet as a whole. Unless an internal water excluding barrier layer is present separating off the first zone, the water activity will equilibrate throughout the tablet to reach the same value throughout.

To improve the separation of the probiotic micro-organisms from the ingredients that are hostile to their stability, said first zone may be separated from said second zone by a water excluding barrier material. Additionally or instead, the tablet as a whole may be surrounded by a water excluding material. Such materials may be cellulose acetate phthalate, methacrylic acid copolymers, alginic acid, zein, modified starch, polyvinylacetate phthallate, hydroxypropylmethylcellulose phthalate, cellulose acetate phthalate, or shellac.

The barrier materials may more preferably be or include a fat based material, which may be applied by a process of hot melt coating. These include but are not limited to fatty acid triglycerides, e.g. hydrogenated palm oil or beef tallow and mixtures of triglyceride esters of higher saturated fatty acids along with varying proportions of mono- and di- glycerides, e.g. hard fats.

Tablets according to the invention may be stored in a container containing a desiccant for absorbing water so as to reduce the water activity in the area surrounding said tablet. Thus, the tablets may be packaged in such a way as to preserve their initial state of dryness within acceptable limits. This may involve packaging the tablets in a moisture impermeable container such as a tube or a blister pack, which may contain a desiccant agent such as silica gel . For protection against oxygen such a pack may contain an oxygen scavenger material such as Amosorb™, ascorbyl palmitate or other ascorbates, propyl galates or other –gallates, alpha-tocopherol, magnesium or sodium sulfite, butylated hydroxyanisole or butylated hydroxytoluene. Oxygen absorbents as described in US-A-5885481, 5744056, or 6083585 can be used.

The tablets may contain additional materials, especially in the second zone, such as plant materials, including herb materials, for example Echinacea, elderberry extract, blueberry extract, cranberry extract and rose hip.

The term 'probiotic micro-organism' is well understood by those skilled in the art to which this invention pertains. Probiotics are micro organisms, which in tablet formulations are normally freeze dried and are normally live, which have a beneficial effect on health when ingested. The probiotic micro-organisms may be lactic acid producing bacteria, e.g. . *Lactobacilli* and *Bifidobacteria* bacteria. Probiotic micro-organisms that may be present include but are not limited to:

*Bibidobacterium*

*-bifidum*
*-longum*
*-adolescentis*
*-animalis*
*-infantis*
*-breve*
*-lactis*

*Lactobacillus*
*-casei*
*-acidophilus*
*-paracacei*
*-plantarum*
*-rhamnosus*
*-reuteri*
*-gasseri*
*-jensenii*
*-delbruekii* including subspecies *delbrueckii* and *bulgaricus*
*-helveticus*
*-salivarius*
*-brevis*
*-johnsonii*
*-crispatus*

*Bacillus*
*-coagulans*

*Saccharomyces*
*-boulaardii*
*-cerevisiae*

*Streptococcus*
*-thermophilus*

*Enterococcus*
*-faecium*
*-faecalis*

*Propionebacterium*
*-freudenreichii*

*Lactococcus*
*-lactis*

*Propionebacterium*
*-freudenreicii*

Each tablet suitably will contain from $10^6$, more preferably from $10^7$ to $10^{12}$, e.g. from $10^8$ to $10^{10}$, viable micro-organism cells.

Preferred methods for producing tablets from the tablet ingredients include standard tabletting methods, including those conventionally used for producing multi-layer tablets. As we have found that excessive tabletting pressure can decrease the viability of the micro-organisms, we prefer that the compression pressure for the probiotic layer should not exceed 50kN/cm$^2$, corresponding to a tensile strength below 100N (Erweka equipment).

The tablets may be designed to be chewed or to be swallowed whole. When the tablets disintegrate on consumption, whether in the mouth or in the stomach, the micro-organisms are exposed to the materials from which they were held separate in the tablet structure. This may harm the micro-organisms if the local concentration of the damaging materials is too high. To guard against this, it is preferred that the disintegration of the two zones or layers be spaced in time to a degree to allow the contents of one zone to be diluted and dispersed before the other zone is released. This may be achieved by the inclusion in one zone or layer of disintegrant agents selected to provide faster disintegration of that zone. The effect may be quantitated by a dissolution test in which a tablet is allowed to disintegrate in unstirred water in a beaker at 25 °C and after one zone has disintegrated, the remainder of the tablet is removed, dried and weighed to establish the amount of that zone of the tablet remaining (as a proportion of the total amount of that zone initially). Preferably, in such a test, the remainder should amount to no less than 20%, more preferably no less than 50%, most preferably no less than 70% of the original amount of that zone or layer.

The test may alternatively be conducted on a time measurement basis in which the tablet is allowed to dissolve as before but the time when a first zone has disintegrated is noted and the time when the total tablet has disintegrated is noted. If both layers disintegrated at the same rate, the first time period would be the same as the total disintegration time. When one zone disintegrates faster, as preferred, the first time period as a percentage of the total disintegration time is preferably no more than 50%, more preferably no more than 20% and most preferably no more than 5% of the total.

Ingredients that promote rapid disintegration (super-disintegrants) that can be included in one of the zones for this purpose include sodium croscarmellose, cross linked sodium carboxymethylcellulose, crospovidone, sodium starch gycolate, sodium starch glyconate and pregelatinized starch.

The invention will be further described with reference to the following illustrative examples of multilayer tablets, containing freeze dried probiotic cultures and vitamins/minerals, herbals or drugs.

Example 1

The following ingredients were formulated into a two layer tasty chewable tablet incorporating lactic acid bacteria, vitamins and minerals using Xylitol and Isomalt to provide bulk and sweetening:

Per tablet:

| | | | |
|---|---|---|---|
| Vitamin A | mcg | 700.00 | Retinolacetate |
| Vitamin D | mcg | 5.00 | Cholecalciferol |
| Vitamin E | IU | 10.43 | D,L-alfa-tocopherolacetate |
| Vitamin B1(salt) | mg | 1.00 | Thiaminenitrate |
| Vitamin B2 | mg | 1.20 | Riboflavin |
| Vitamin B6(salt) | mg | 1.10 | Pyridoxine chloride |
| Vitamin B12 | mcg | 1.40 | Cyanocobalamin |
| Nicotinamide | mg | 13.00 | Nicotinamide |
| Pantothenic acid | mg | 5.00 | D-Calcium pantothenate |
| Folic acid | mcg | 100.00 | Folic acid |
| Vitamin C | mg | 60.00 | Ascorbic acid |
| Calcium | mg | 200.00 | Calcium carbonate |
| Magnesium | mg | 50.00 | Magnesium oxide |
| Iron | mg | 10.00 | Ferrous fumarate |
| Zinc | mg | 7.00 | Zinc oxide |
| Copper | mg | 0.70 | Cupric oxide |
| Manganese | mg | 2.00 | Manganese sulfate |
| Chromium | mcg | 50.00 | Chromium (III) chloride |
| Selenium | mcg | 30.00 | Sodium selenate |
| Iodine | mcg | 90.00 | Potassium iodide |
| Biotin | mcg | 30.00 | d-Biotin |

| | | | |
|---|---|---|---|
| Vitamin K | mcg | 30.00 | Phytomenadione |
| Lactobacillus GG cfu | $1x10^9$ | | |

The vitamins and minerals (except for selenium) are mixed with the following excipients:

| | |
|---|---|
| Xylitol | 320 mg |
| Microcrystalline cellulose | 64 mg |
| Flavour | 33 mg |
| Stearic acid | 22 mg |
| Silicon dioxide | 7 mg |
| Acesulfam potassium | 2 mg |
| (in total | 700 mg) |

The freeze dried probiotic culture (10 mg = $3x10^9$) and the selenium is mixed with:

| | |
|---|---|
| Isomalt | 253 mg |
| Xylitol | 100 mg |
| Microcrystalline cellulose | 31 mg |
| Magnesium stearate | 4 mg |
| silicon dioxide | 2 mg |
| (in total | 400 mg) |

Tablets were produced having two superposed layers using a conventional tabletting machine, the ingredients of one layer being filled over the ingredients of the other.

| | |
|---|---|
| Tablet weight | 1100 mg |
| Tablet size | 11 by 16.5 mm oval |
| Water activity** in culture granulate | <0.1 |
| Water content* in culture granulate | 2% |
| Water activity** in tablet | 0.09 |
| Water content* in tablet | 2.7% |

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For comparison, a single layer tablet was produced containing the same ingredients. The viability of the micro-organisms was measured after storage of the tablets over nine months with the following results:

| Months | Single layer tablet | Dual layer tablet |
|---|---|---|
| 0 | $7.3 * 10^8$ | $1.5 * 10^9$ |
| 1.5 | $6.9 * 10^7$ | $1.1 * 10^9$ |
| 6 | $1.5 * 10^7$ | $3.4 * 10^7$ |
| 9 | $< 2 * 10^3$ | $1.1 * 10^5$ |

It can be seen that the two layer tablet of the invention maintained the viability of the micro-organisms over the total storage period better by a factor of over 100.

Example 2

The following ingredients were formulated as a two layer tablet to swallow with lactic acid bacteria, vitamins and minerals.

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Per tablet:

| | | | |
|---|---|---|---|
| Vitamin D | mcg | 5.00 | Cholecalciferol |
| Vitamin E | IU | 14.90 | D,L-alfatocopherolacetate |
| Vitamin B1(salt) | mg | 5.00 | Thiaminenitrate |
| Vitamin B2 | mg | 5.00 | Riboflavin |
| Vitamin B6(salt) | mg | 5.00 | Pyridoxinchloride |
| Vitamin B12 | mcg | 3.00 | Cyanocobalamin |
| Biotin | mcg | 30.00 | d-Biotin |
| Nicotinamide | mg | 18.00 | Nicotinamide |
| Pantothenic acid | mg | 5.00 | D-Calciumpantothenate |
| Folic acid | mcg | 400.00 | Folic acid |
| Vitamin C | mg | 90.00 | Ascorbic acid |
| Magnesium | mg | 90.00 | Magnesium oxide |
| Zinc | mg | 15.00 | Zinc oxide |
| Manganese | mg | 2.50 | Manganese sulfate |
| Chromium | mcg | 30.00 | Chromium (III) chloride |
| Selenium | mcg | 50.00 | Sodium selenate |
| Iodine | mcg | 100.00 | Calcium iodide |

Lactobacillus GG cfu $1x10^9$

The vitamins and minerals (except for selenium) are mixed with the following excipients:

| | |
|---|---|
| Microcrystalline cellulose | 58 mg |
| Magnesium stearate | 4 mg |
| Stearic acid | 3 mg |
| Silicon dioxide | 1 mg |
| (in total | 555 mg) |

The freeze dried probiotic culture (10 mg = $3x10^9$) and the selenium are mixed with:

| | |
|---|---|
| Microcrystalline cellulose | 183 mg |
| Magnesium stearate | 2 mg |
| Silicon dioxide | 0.4 mg |
| (in total | 195 mg) |

Tabletting was conducted as in Example 1 and the 2-layer tablets were filled into aluminium tubes with desiccant in the lid.

| | |
|---|---|
| Tablet weight | 750 mg |
| Tablet size | 12 by 4 mm circular |
| Water activity** in culture granulate | 0.07 |
| Water content* in culture granulate | 2% |
| Water activity** in tablet | 0.07 |
| Water content* in tablet | 3.2% |

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Example 3

The following ingredients were formulated into a two layer tasty chewable tablet incorporating lactic acid bacteria, vitamins and minerals using Xylitol and Lactitol to provide bulk and sweetening:

Per tablet:

| | | | |
|---|---|---|---|
| Vitamin A | mcg | 700.00 | Retinolacetate |
| Vitamin D | mcg | 5.00 | Cholecalciferol |
| Vitamin E | IU | 10.43 | D,L-alfa-tocopherol acetate |
| Vitamin B1(salt) | mg | 1.00 | Thiaminenitrate |
| Vitamin B2 | mg | 1.20 | Riboflavin |
| Vitamin B6(salt) | mg | 1.10 | Pyridoxine chloride |
| Vitamin B12 | mcg | 1.40 | Cyanocobalamin |
| Nicotinamide | mg | 13.00 | Nicotinamide |
| Pantothenic acid | mg | 5.00 | D-Calcium pantothenate |
| Folic acid | mcg | 100.00 | Folic acid |
| Vitamin C | mg | 60.00 | Ascorbic acid |
| Calcium | mg | 200.00 | Calcium carbonate |
| Magnesium | mg | 50.00 | Magnesium oxide |
| Iron | mg | 10.00 | Ferrous fumarate |
| Zinc | mg | 7.00 | Zinc oxide |
| Copper | mg | 0.70 | Cupric oxide |
| Manganese | mg | 2.00 | Manganese sulfate |
| Chromium | mcg | 50.00 | Chromium (III) chloride |
| Selenium | mcg | 30.00 | Sodium selenate |
| Iodine | mcg | 90.00 | Potassium iodide |
| Biotin | mcg | 30.00 | d-Biotin |
| Vitamin K | mcg | 30.00 | Phytomenadione |
| Lactobacillus GG | cfu | $1x10^9$ | |

The vitamins and minerals (except for selenium) are mixed with the following excipients:

| | |
|---|---|
| Lactitol | 209 mg |
| Microcrystalline cellulose | 39 mg |
| Flavour | 2.5 mg |
| Stearic acid | 44 mg |
| Silicon dioxide | 14 mg |
| Neohesperidin 10% | 0.2 mg |
| Citric acid monohydrate | 2 mg |

(in total 1160 mg)

The freeze dried probiotic culture (10 mg = $3x10^9$) and the selenium is mixed with:

| | |
|---|---|
| Lactitol | 394 mg |
| Microcrystalline cellulose | 21 mg |
| Stearic acid | 14 mg |
| (in total | 440 mg) |

Tabletting was conducted as in Example 1 and the 2-layer tablets were filled into aluminium tubes with desiccant in the lid.

| | |
|---|---|
| Tablet weight | 1600 mg |
| Tablet size | 16 mm circular |
| Water activity** in culture granulate | <0.1 |
| Water content* in culture granulate | 3.1% |
| Water activity** in tablet | 0.09 |
| Water content* in tablet | 3.7% |

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The tablets were tested for stability by storage for 18 months in higher (24%), middle (20%) and lower (7%) relative humidity conditions and viability of the micro-organisms was monitored, with the following results:

| Months | 24% humidity | 20% humidity | 7% humidity |
|---|---|---|---|
| 0 | $1.7 * 10^9$ | $1.7 * 10^9$ | $1.7 * 10^9$ |
| 6 | $8.7 * 10^6$ | $1.3 * 10^8$ | $0.9 * 10^9$ |
| 9 | $8.5 * 10^5$ | $8.0 * 10^8$ | $0.6 * 10^9$ |
| 12 | $1.4 * 10^4$ | $3 * 10^6$ | $0.6 * 10^9$ |
| 18 | $< 2 * 10^3$ | $5.7 * 10^5$ | ND |

Thus, it can be seen that the tablets of the invention provided excellent long term stability.

In the above Examples, the vitamins used were in some cases supplied in an encapsulated form, others were used in non-encapsulated form. The table below indicates the ingredients present in the vitamin formulations used

| **Active ingredients** | **Amount** |
|---|---|
| **Vitamin D (Cholecalciferol)** | **5 mcg = 200 IU** |
| As Cholecalciferol Concentrate Powder (analysed to 110 IU/mg) | 2.00 mg |
| - Cholecalciferol | 6 mcg |
| - Sucrose | 0.68 mg |
| - Gelatin | 0.42 mg |
| - Modified Starch | 0.42 mg |
| - Triglycerides, medium-chain | 0.38 mg |
| - Butyl Hydroxytoluene | 19 mcg |
| - Sodium Aluminosilicate<br>- Water | 3 mcg<br>72 mcg |
| **Vitamin E (D-α-tocopherol)** | **14.90 IU** |
| As α-Tocopherol Acetate Concentrate (Powder form)(analysed to 52,5 w/w %) | 30.08 mg |
| - DL-α-Tocopherol Acetate | 15.79 mg |
| - Maize Starch | 6.02 mg |
| - Gelatin | 5.11 mg |
| - Sucrose | 1.41 mg |
| - Sodium Aluminosilicate | 0.39 mg |
| - Water | 1.35 mg |
| **Vitamin B1 (Thiamin)** | **5 mg** |
| As Thiamin Nitrate 33% | 14.85 mg |
| - Thiamin nitrate | 4.95 mg |
| - Mixture of mono-, di and triglycerides | 9.90 mg |
| **Vitamin B2 (Riboflavin)** | **5 mg** |

| **Active ingredients** | **Amount** |
|---|---|
| As Riboflavine 33% | 15.60 mg |
| - Riboflavine | 5.20 mg |
| - Mixture of mono-, di and triglycerides | 8.84 mg |
| - Maize Starch | 1.56 mg |
| **Vitamin B6 (Pyridoxine)** | **5 mg** |
| As Pyridoxine Hydrochloride 33% | 15.45 mg |
| - Pyridoxine Hydrochloride | 5.15 mg |
| - Mixture of mono-, di and triglycerides | 10.30 mg |
| **Vitamin B12** | **3 mcg** |
| As Cyanocobalamine 0.1% (analysed to 0.11%) | 1.87 mg |
| - Cyanocobalamine | 3 mcg |
| - Maltodextrin | 2.64 mg |
| - Sodium citrate<br>- Citric acid<br>- Water | 27 mcg<br>20 mcg<br>120 mcg |
| **Biotin** | **30 mcg** |
| As D-Biotin | 32 mcg |
| **Nicotinamide** | **18 mg** |
| As Nicotinamide 33% | 56.16 mg |
| - Nicotinamide | 18.72 mg |
| - Mixture of mono-, di and triglycerides<br>- Silicon dioxide | 31.82 mg<br>5.62 mg |
| **Pantothenic Acid** | **5 mg** |
| As Calcium Pantothenate | 5.56 mg |
| **Folic Acid** | **400 mcg** |
| Folic Acid | 0.49 mg |
| - Folic Acid | 0.44 mg |
| - Absorbed Water | 49 mcg |

| **Active ingredients** | **Amount** |
|---|---|
| **Vitamin C (Ascorbic Acid)** | **90 mg** |
| As Ascorbic Acid 97% | 100.21 mg |
| - Ascorbic Acid | 97.20 mg |
| - Maize Starch | 3.01 mg |
| **Vitamin A (Retinol)** | **700 mcg** |
| Vitamin A Concentrate Synthetic (Powder form)(analysed to 565 IU/mg) | 5.21 mg |
| - Retinol Acetate | 1.02 mg |
| - Sucrose | 1.77 mg |
| - Gelatin | 1.25 mg |
| - Modified Starch | 0.83 mg |
| - Butylated Hydroxytoluene | 0.07 mg |
| - Sodium Aluminosilicate | 18 mcg |
| - Water | 0.25 mg |

Example 4

Effect of selenium on viability on storage:

The following mixtures have been stored in a dehumidified room at a temperature of 25 °C. Starting counts and counts of viable organisms after the indicated storage period were measured.

(a)

5mg LGG + 295mg Microcrystalline cellulose:

Start week 0: count $3,5x10^9$ Cfu/ tablet

End week 8: count $2,9x10^9$ Cfu/tablet

(b)

5mg LGG + 0.05mg Selenium + 295mg Microcrystalline cellulose:

Start week 0: count $4,0 x10^9$ Cfu/ tablet

End week 8: $4,6x10^9$ Cfu/tablet

It can be seen that the presence of selenium was beneficial to the stability of the micro-organisms, and indeed that the numbers of recoverable micro-organisms even increased on storage in the presence of selenium.

In each case the probiotic bacteria were *Lactobacillus rhamnosus GG* "Grade P" (ATCC 53103) as a concentrated, freeze-dried bacterial powder.

Example 5 – tablets with differential speed of disintegration of layers

The composition of the probiotic layer, but not of the vitamin/mineral layer, of the tablet of Example 2 was modified in three ways as follows:

Freeze dried probiotic culture and selenium - unchanged

Probiotic layer formulation (a)

| | |
|---|---|
| Selenium granulate 2% | 2.5mg |
| Silicon dioxide | 0.4mg |
| Lactose anhydrous | 181mg |
| Magnesium stearate | 1.5mg |

Probiotic layer formulation (b)

| | |
|---|---|
| Selenium granulate 2% | 2.5mg |
| Silicon dioxide | 0.4mg |
| Lactose anhydrous | 171.7mg |
| Croscarmellose sodium | 1.5mg |

Probiotic layer formulation (c)

| | |
|---|---|
| Selenium granulate 2% | 2.5mg |
| Silicon dioxide | 0.4mg |
| Lactose anhydrous | 171.7mg |
| Magnesium stearate | 1.5mg |
| Povidone | 9.3mg |

The dissolution time of the two layers was measured in each case by observing disintegration of the tablet in a beaker of water with the following results:

Vitamin/mineral layer: 14 minutes

Probiotic layer:

Formulation (a) 6 minutes

Formulation (b) 1 minute 45 sec

Formulation (c) 15 sec

Example 6 – further tablets with differential speed of disintegration of layers

A two layer tablet was produced in which a probiotic containing layer was formulated as follows:

The freeze dried probiotic culture (10 mg = 3x109) is mixed with:

| | |
|---|---|
| Selenium Granulate 2% | 2.5 mg |
| Silicon Dioxide | 0.8 mg |
| Magnesium Stearate | 1.5 mg |
| Cellulose, Microcrystalline Cellulose | 152.4 mg |
| Hypromellose 15000 | 27.8 mg |

The vitamin/mineral layer was as from example 2 with either 0% Croscarmellose (Formulation 1)
or 5% Croscarmellose Sodium (Formulation 2)

In a dissolution test conducted as above, the results were as follows:

Disintegration time

Probiotic layer 10 minutes

Vitamin/mineral layer
1: 37 minutes
2: 3 minutes